JHR 98: 1087–1106 (2025)
doi: 10.3897/jhr.98.170415
https://jhr.pensoft.net

RESEARCH ARTICLE

JOURNAL OF Hymenoptera RESEARCH
A peer-reviewed open-access journal
The International Society of Hymenopterists

Vibroacoustic signalling of the Maltese *Messor* ants (Hymenoptera, Formicidae), with geographical insights into *M. ibericus*

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Academic editor: Francisco Hita Garcia | Received 28 August 2025 | Accepted 24 October 2025 | Published 17 November 2025

https://zoobank.org/B3C7233E-6A66-4D1E-BC1B-954DAF98404D

Citation: Lapeva-Gjonova A, Gjonov I, Kostova R, Mifsud D (2025) Vibroacoustic signalling of the Maltese *Messor* ants (Hymenoptera, Formicidae), with geographical insights into *M. ibericus*. Journal of Hymenoptera Research 98: 1087–1106. https://doi.org/10.3897/jhr.98.170415

Abstract

This study presents vibroacoustic recordings of the three *Messor* species in the Maltese archipelago, *M. capitatus* (Latreille, 1798), *M. bouvieri* Bondroit, 1918, and *M. ibericus* Santschi, 1925, and their signal characteristics were analysed. The observed patterns are consistent with the hypothesis that acoustic traits in *Messor* ants are subject to species-specific constraints. To gain further insight into the sound parameters of *M. ibericus*, geographically isolated populations from Malta, Gozo, Sicily, Crete, and Bulgaria were compared, revealing variation and significant differences at the population level. Additionally, the study provides new occurrence data for *M. ibericus* (queens and workers), which was previously reported as *M. structor* (Latreille, 1798) for Malta, along with information on its habitat preferences and activity.

Keywords

Biogeography, harvester ants, Mediterranean, vibroacoustics

Introduction

Over the last decades, increasing information has accumulated on the unexpectedly wide distribution of hidden taxonomic diversity in European ants (Seifert 2009; Csősz et al. 2014; Seifert 2018; Steiner et al. 2018). In this context, one of the emblematic examples in this line are the ants of the genus *Messor* Forel, 1890, known as harvester

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ants, in which intraspecific variability, polymorphism, cases of hybridisation and even xenoparity are well documented (Schlick-Steiner et al. 2006; Steiner et al. 2011, 2018; Orou et al. 2023; Juvé et al. 2025a, 2025b).

The application of complementary analyses from the genetics, cuticular hydrocarbons, ethology and ecology to standard comparative morphology and morphometrics in ants, known as integrative taxonomy, is the most convincing approach to reveal true biodiversity (Seifert 2009). In recent years, acoustic communication studies in a number of ants have shown that they can often be species-specific and has been successfully used to detect and confirm new species, especially those that are morphologically indistinguishable from other closely related species (Pavan et al. 1997; Ferreira et al. 2010; Peña Carrillo 2021).

Species of *Messor* are known to possess stridulatory organs that produce vibrational signals by rubbing the tergal portion of the postpetiolus, known as the scraper (or *plectrum*), against a finely striated region called the file (or *pars stridens*), located dorsally to the first metasomal segment (Grasso et al. 1998; Hernández et al. 2002). However, only in a few species stridulation fields (SF) have been studied and documented by scanning electron microscopy. Even more limited are available records of the acoustic signals produced and their role in behaviour (e.g. Hahn and Maschwitz 1985; Priano et al. 1997; Grasso et al. 2009). Vibroacoustic signals have been analysed in a few species of the genus *Messor*, including *M. barbarus* (Linnaeus, 1767), *M. capitatus* (Latreille, 1798), *M. minor* (André, 1883), *M. structor* (Latreille, 1798), and *M. wasmanni* Krausse, 1910 (Grasso et al. 1999, 2000; Gjonov et al. 2025).

The majority of species in the genus *Messor* occur in the Palaearctic, with the Mediterranean subregion having high species diversity (Borowiec 2014). The historical accumulation of many synonyms and species names makes the genus taxonomically challenging, and recent taxonomic revisions are available for two of the species groups, *M. structor* and *M. semirufus* (Steiner et al. 2018; Salata et al. 2023).

To date, three species of the genus *Messor* have been documented from the Maltese archipelago, recorded from a considerable number of localities. These include *Messor bouvieri* Bondroit, 1918, *Messor capitatus*, and *M. structor*. A fourth species of the genus from the island of Comino was reported by Baroni Urbani (1968) and Schembri and Collingwood (1981) and identified as *M. meridionalis* ssp. *wasmanni* Krausse and *M. caducus* (Victor, 1839). In the Mediterranean Region, both taxa are now recognised as *M. wasmanni*. However, the presence of this species in Malta raise questions about its natural occurrence, particularly given the absence of this species from the islands of Malta, Gozo, and Sicily (Baroni Urbani 1968).

This study presents vibroacoustic recordings of all three *Messor* species typical of the Maltese archipelago, *M. capitatus*, *M. bouvieri*, and *M. ibericus* Santschi, 1925, the latter previously reported as *M. structor* for this region. In order to gain further insight into the sound parameters of workers of the species *M. ibericus*, a comparison between geographically isolated populations from Malta, Gozo, Sicily, Crete and Bulgaria was conducted. In addition, new occurrence data of *M. ibericus* from Malta and Gozo are provided. Notably, a recent discovery indicates that *M. ibericus* workers are hybrids

derived from queens of this species and males of *M. structor* (either wild-type or clonal lineages), even though *M. structor* itself may be absent from the area, as has been demonstrated for Sicily (Juvé et al. 2025a).

Materials and methods

Samples collection

Specimens of *Messor* species were collected by hand throughout the islands of Malta and Gozo to assess species occurrence within the archipelago and were stored in 96% ethanol for morphological examination. They were sampled by the first author in 2017 (November), 2024 (April) and 2025 (March). Identification of the ants follow Schembri and Collingwood (1981) and Seifert (2018).

Specimens used for vibroacoustic recordings were collected in Malta (April 2024), Crete (September 2023), Bulgaria (May 2024), and Sicily (May 2025). They were kept alive until processing, which was carried out indoors. The studied material is stored in the Zoological collection of Sofia University (BFUS). Data on the occurrence by species and nesting samples of the specimens used for sound recordings are provided below, and additional details on the presence of *M. ibericus* in Malta are provided in Suppl. material 1. Considering the genetic complexity observed in *M. ibericus* (including the hybrid origin of workers) by Juvé et al. (2025a), we use this name for the worker caste as well, for consistency. Although we acknowledge that the worker caste represents hybrid individuals (*M. ibericus* × *M. structor*), our study is not genomic or taxonomic in scope, and therefore the use of the established species name ensures comparability with previous ecological and morphological research.

***Messor ibericus* Santschi, 1925**
Fig. 1A, B

Sound recordings. Malta – **Malta isl.** • Gnejna Bay; 35°55.224'N, 14°20.640'E; alt. 9 m; 18 Apr. 2024; sound records: R264, R265, R266 (major workers); – **Gozo isl.** • Ta' Pinu; 36°03.630'N, 14°12.546'E; alt. 127 m; 20 Apr. 2024; sound records: R285, R288, R289 (major workers), R287 (queen); Bulgaria • Plovdiv; 42°8.820'N, 24°45.096'E; alt. 205 m; 27 May 2024; sound records: R314, R315, R316 (major workers); Greece • Crete; Thronos; 35°16.188'N, 24°37.086'E; alt. 372 m; 19 Sep. 2023, sound records: R161, R164, R166 (major workers); Italy • Sicily; Vizzini; 37°8.572'N, 14°44.682'E; alt. 395 m; 10 May 2025; sound records: R01, R02, R06 (major workers).

***Messor bouvieri* Bondroit, 1918**
Fig. 1C

Sound recordings. Malta – **Malta isl.** • Ghadira; 35°58.157'N, 14°20.960'E; 4 m; 19 Apr. 2024; sound records: R276, R278, R279 (major workers).

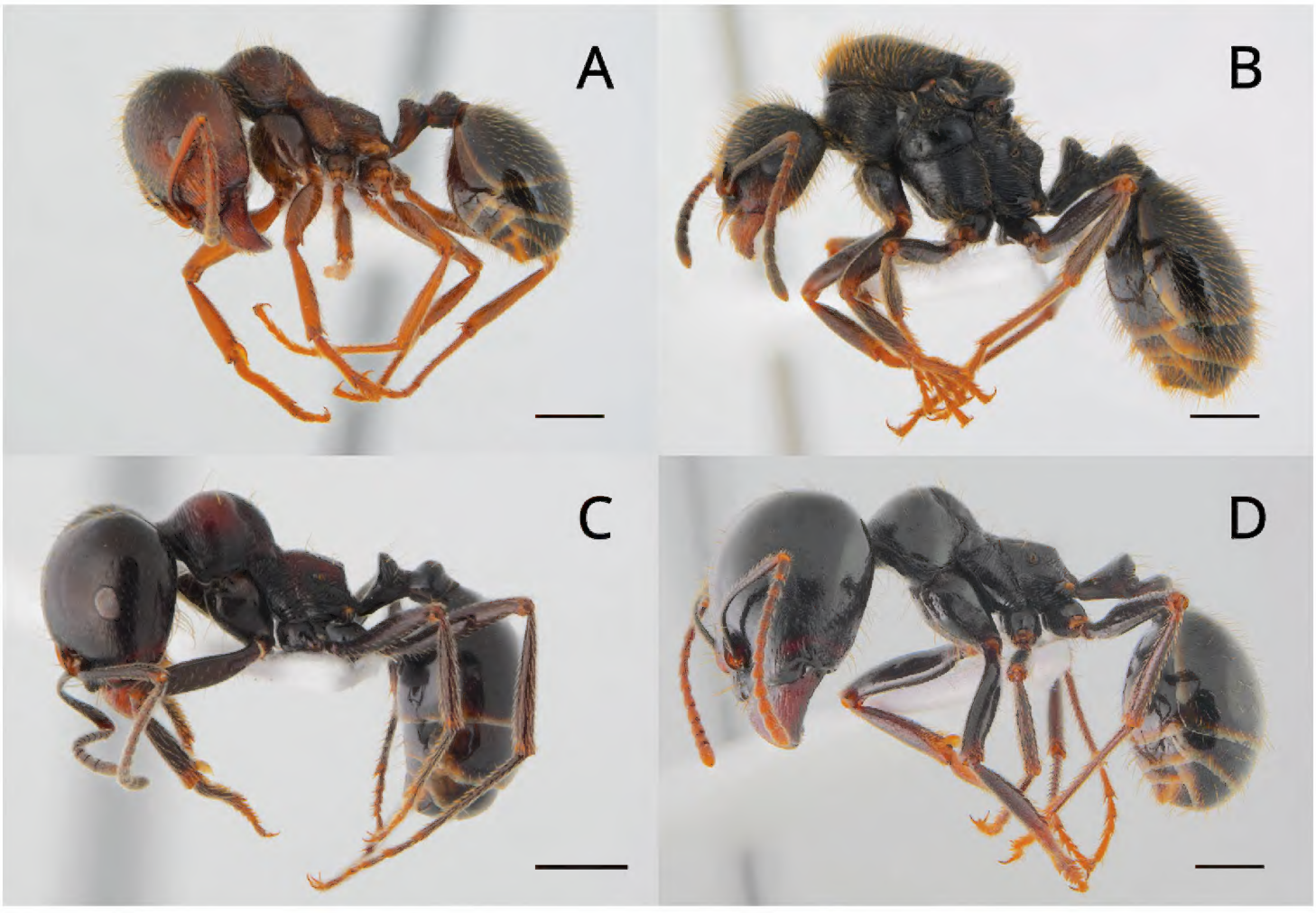

Figure 1. Habitus of Maltese *Messor* ants **A** *Messor ibericus* (worker) **B** *M. ibericus* (queen) **C** *M. bouvieri* (worker) **D** *M. capitatus* (worker). Scale bars: 1 mm.

***Messor capitatus* (Latreille, 1798)**
Fig. 1D

Sound recordings. Malta – **Malta isl.** • Il-Kalanka; 35°49.483'N, 14°33.388'E, 18 m; 16 Apr. 2024; sound records: R242, R243, R248 (major workers).

Photographic images

Images of the specimens were taken with Nikon DS-Ri2 through Nikon SMZ 1270i stereomicroscope and then aligned and stacked using Helicon Focus 8.3.6. Pro. Stridulation fields were imaged by scanning electron microscope JSM-5510 at 10 kW.

Sound recording and analysis

The terminology in Grasso et al. (2000) was used for signal parameters. The recorded signal (presumed disturbing) consists of trains of chirps, each composed of two parts (forward and backward). The maximum frequency was measured not across the entire chirp, but separately for each part. Since the experimental setup did not allow for calibrated measurement of absolute amplitude values, the ratio between measured amplitudes under constant conditions was used for the purposes of this study.

The following signal parameters were selected for comparing acoustic signals: chirp rate (chirps/s), chirp duration (ms), forward chirp duration (ms), backward chirp duration (ms), maximum frequency of the forward chirp (Hz), maximum frequency of the backward chirp (Hz), and the ratio of the amplitude at maximum frequency between the forward and backward parts of the chirp.

Signals were recorded using a DIY piezoelectric contact microphone, following the methodology described by Gjonov et al. (2025), and a Zoom F3 digital recorder. GNU Audacity software was used to process and measure the parameters. Noise in the recordings was suppressed by 24 dB, using a noise sample taken from parts of the recording where no signal was present. After confirming that no recorded signals were within this frequency range, a high-pass filter at 100 Hz with a roll-off of 24 dB per octave was also applied. For each recording, a section containing a relatively long chirp train was selected. Its duration was measured in seconds, and the chirps were counted to obtain the chirp rate value. The other parameters were measured from three chirps within this section.

For each of the three *Messor* species, across the major worker caste and different locations, recordings from three specimens were analysed, except for the queen of *M. ibericus*, for which only a single specimen was available; its measurements are presented but were not included in the analysis. Selected recordings are included in the Suppl. materials 2–5. Sample oscillograms and spectrograms of trains and chirps were prepared for each species, caste, and location.

Statistics

Principal Component Analysis (PCA) was used to visualise the variance among the samples and to reduce the dimensionality of the dataset. Variables with correlations to the axes greater than $r = 0.2$ were retained in the analysis.

Since most of the measured acoustic variables did not follow a normal distribution, a non-parametric Permutational Multivariate Analysis of Variance (PERMANOVA) was employed, incorporating a Monte Carlo permutation procedure appropriate for small sample sizes. Two sets of PERMANOVAs were conducted to assess both overall and pairwise differences in acoustic characteristics: (1) between *Messor* ant species found in Maltese Islands, and (2) among five geographically distant populations of *Messor ibericus* (Malta and Gozo, Sicily, Crete, Bulgaria).

All data were z-score standardised to account for differences in scale and units. Analyses were based on an Euclidean distance matrix, with an unrestricted permutation of raw data—suitable for single-factor designs—as the permutation method. A total of 9999 permutations were performed. To test for differences in variation around group centroids, a homogeneity of multivariate dispersions test (PERMDISP) was applied, as variation in dispersion can influence PERMANOVA results. All groups showed significant differences ($p < 0.01$). Nevertheless, as PERMANOVA is robust to heterogeneity in balanced designs (i.e., equal sample sizes), the results were interpreted with caution (Anderson and Walsh 2013).

To identify the most influential variables distinguishing the groups and for classification purposes, a Stepwise Discriminant Analysis (SDA) was performed. At each step,

the variable that minimised the overall Wilks' lambda—the proportion of total variance in discriminant scores not explained by group differences—was included. Variables were entered into the model if their F-value significance was ≤ 0.05 and removed if ≥ 0.10. A cross-validation procedure was also performed.

The statistical analyses were conducted using PRIMER v7 (Clarke and Gorley 2015) and SPSS 17 (IBM 2007).

Results

Sound recordings – stridulation, oscilograms and spectograms, acoustic characteristics

The stridulatory apparatus of *Messor* species is positioned on the dorsal surface of the postpetiole and the first metasomal tergite (Fig. 2A–D). The *pars stridens*, located in the mid-dorsal region of the first metasomal pretergite, is distinctly oval. Except *M. bouvieri*, the stridulatory field (SF) is wider than long, with transverse parallel ridges that become denser posteriorly. The surrounding tegument displays an irregular strigulate texture of short transverse ridges, either convex or concave. Well-developed "pillars"

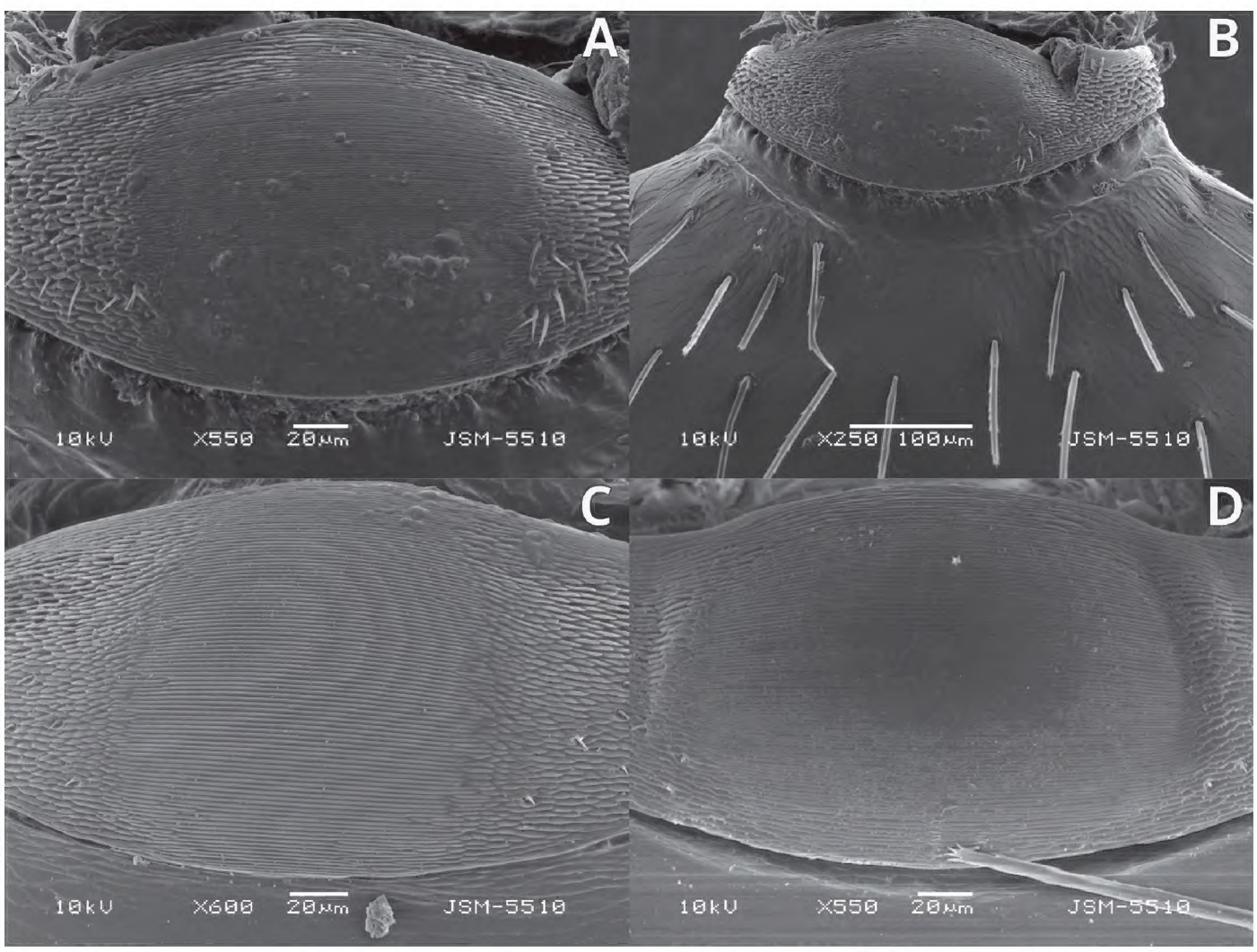

Figure 2. Stridulatory fields in Maltese *Messor* ants **A, B** *M. ibericus* (worker) **C** *Messor bouvieri* (worker) **D** *M. capitatus* (worker).

beneath the pretergite margin are clearly visible in SEM images of *M. ibericus*, while in *M. capitatus* the *pars stridens* is sharply delimited from the adjacent integument.

When restrained, ants emit stridulatory signals through characteristic dorsoventral abdominal movements, produced either in brief bursts or in extended sequences. The resulting vibrations are transmitted via the pincer. A single stroke of the *plectrum* across the stridulatory field generates a series of pulses, termed "chirps," each arising from contact between the *plectrum* and an individual ridge. In *Messor*, the sequential passage of the *plectrum* produces a distinctive disyllabic chirp.

Oscillograms and spectrograms of portions of trains and chirps from the vibroacoustic signals of major workers of *Messor bouvieri*, *M. capitatus*, and *M. ibericus* are presented below (Figs 3–6), and audio files, after noise reduction and high-pass filtering, are also provided (see Suppl. materials 2–5). For *M. ibericus*, recordings were obtained

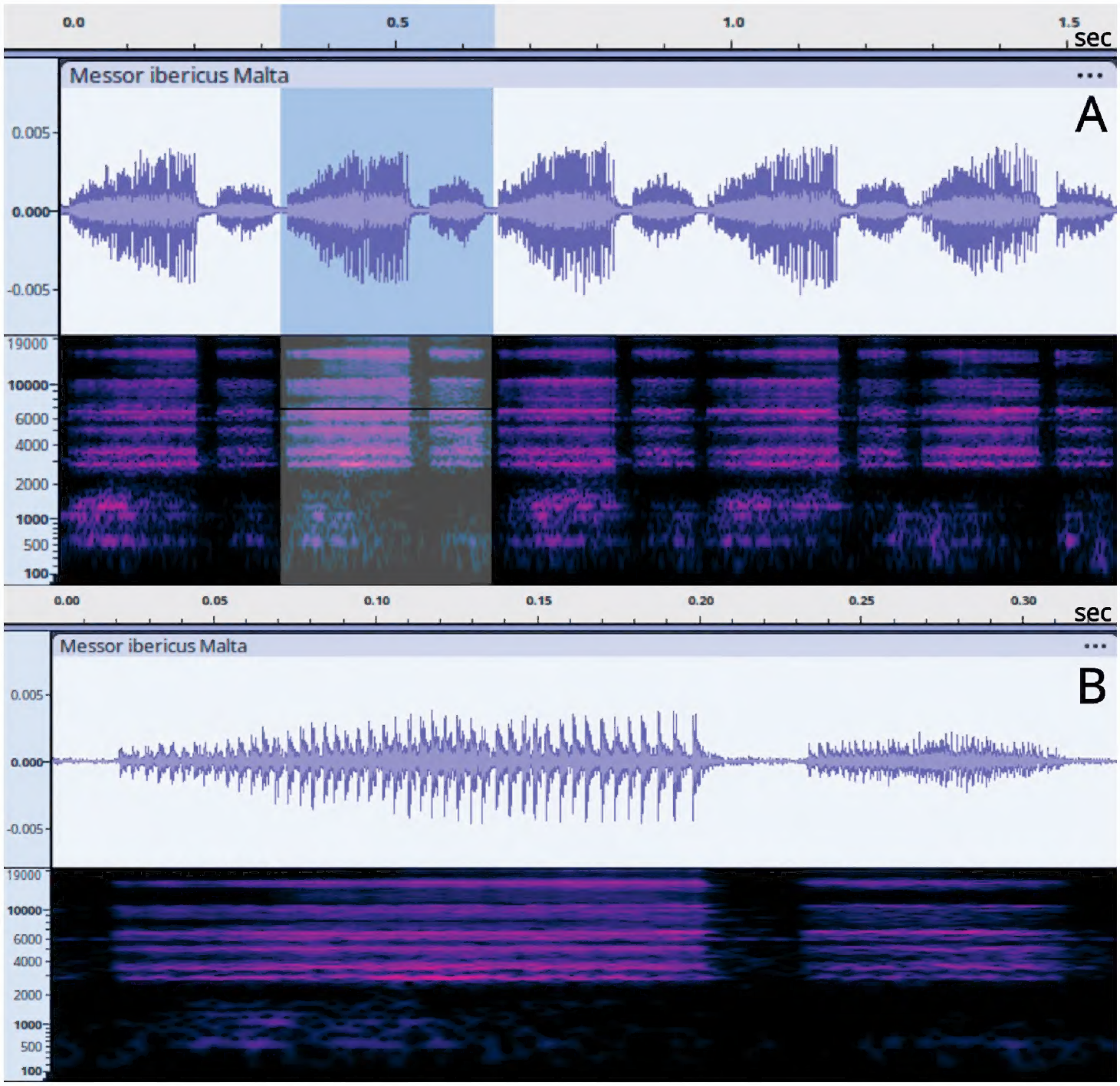

Figure 3. Oscillogram and sonogram of stridulation distress signals in *Messor ibericus* (worker) **A** train **B** chirp.

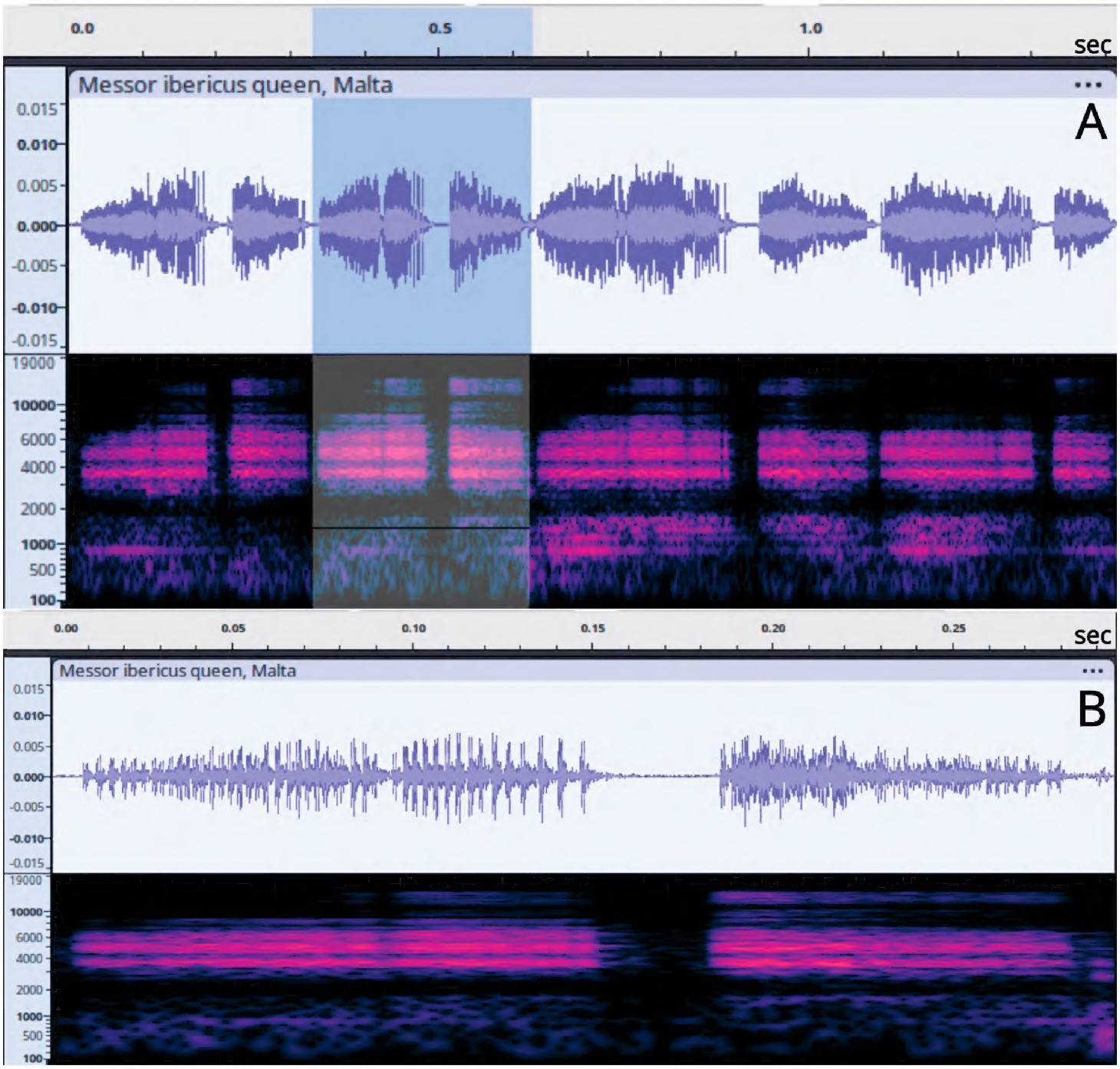

Figure 4. Oscillogram and sonogram of stridulation distress signals in *Messor ibericus* (queen) **A** train **B** chirp.

from a queen collected on the island of Gozo (Fig. 4) as well as from major workers sampled across different populations, with material from Malta, Sicily, Crete, and Bulgaria.

Acoustic characteristics of the three *Messor* species found in Malta

Selected parameters of acoustic signals were used to characterise the three species of the genus *Messor* found on the Maltese Islands (Table 1).

PERMANOVA results indicated significant differences in the acoustic characteristics of the three Maltese *Messor* species ($F = 15.96$, $p(MC) < 0.001$), with post-hoc pairwise tests confirming differences between all species pairs (Suppl. material 6: tables 1, 2). The separation was clearly evident in the PCA plot (Fig. 7), where the first two axes explained 84.7% of the total variation. SDA identified chirp duration, chirp rate,

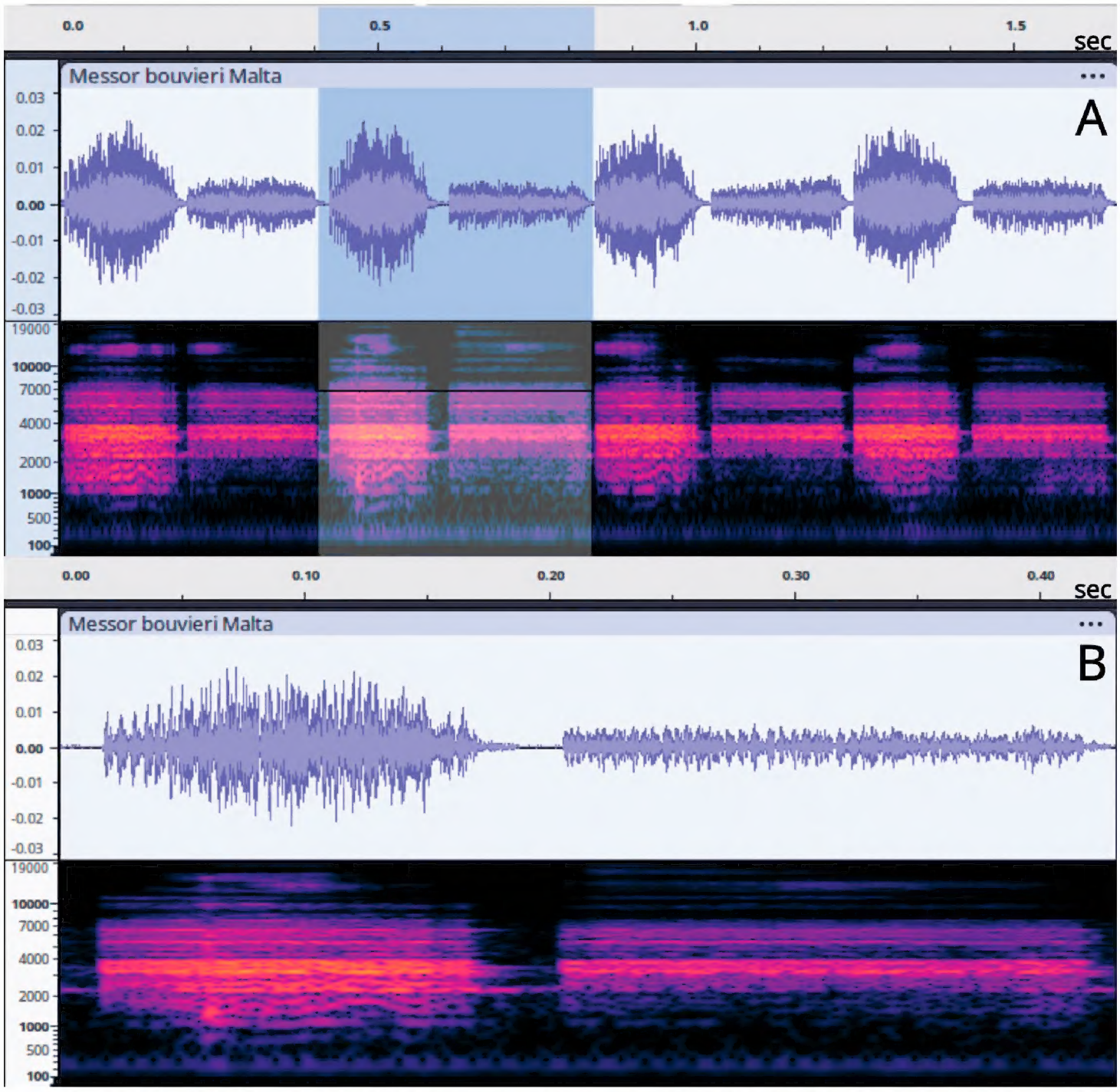

Figure 5. Oscillogram and sonogram of stridulation distress signals in *Messor bouvieri* (worker) **A** train **B** chirp.

the amplitude ratio between the forward and backward chirp and backward chirp duration as the most important discriminative variables. Cross-validation achieved 100% classification accuracy (Suppl. material 6: tables 3, 4). Although the sample size was relatively small and may not capture the full range of variation in population-level acoustic traits, distinct species-specific patterns were apparent. *M. bouvieri* exhibited the longest chirp duration (428.22 ms), the slowest chirp rate (2.31 chirps/s), and the highest forward-to-backward chirp amplitude ratio (1.17), followed by *M. capitatus* and *M. ibericus* which demonstrated the highest chirp rate of 2.98 chirps/s (Table 1). Both *M. bouvieri* and *M. capitatus* had a longer backward chirp, whereas *M. ibericus* showed a longer forward chirp. *M. capitatus* had the highest frequencies in both chirp parts (forward – 3777.89 Hz and backward – 3723.44 Hz), while *M. bouvieri* and *M. ibericus* had lower and more similar frequencies (Table 1). The lowest overall

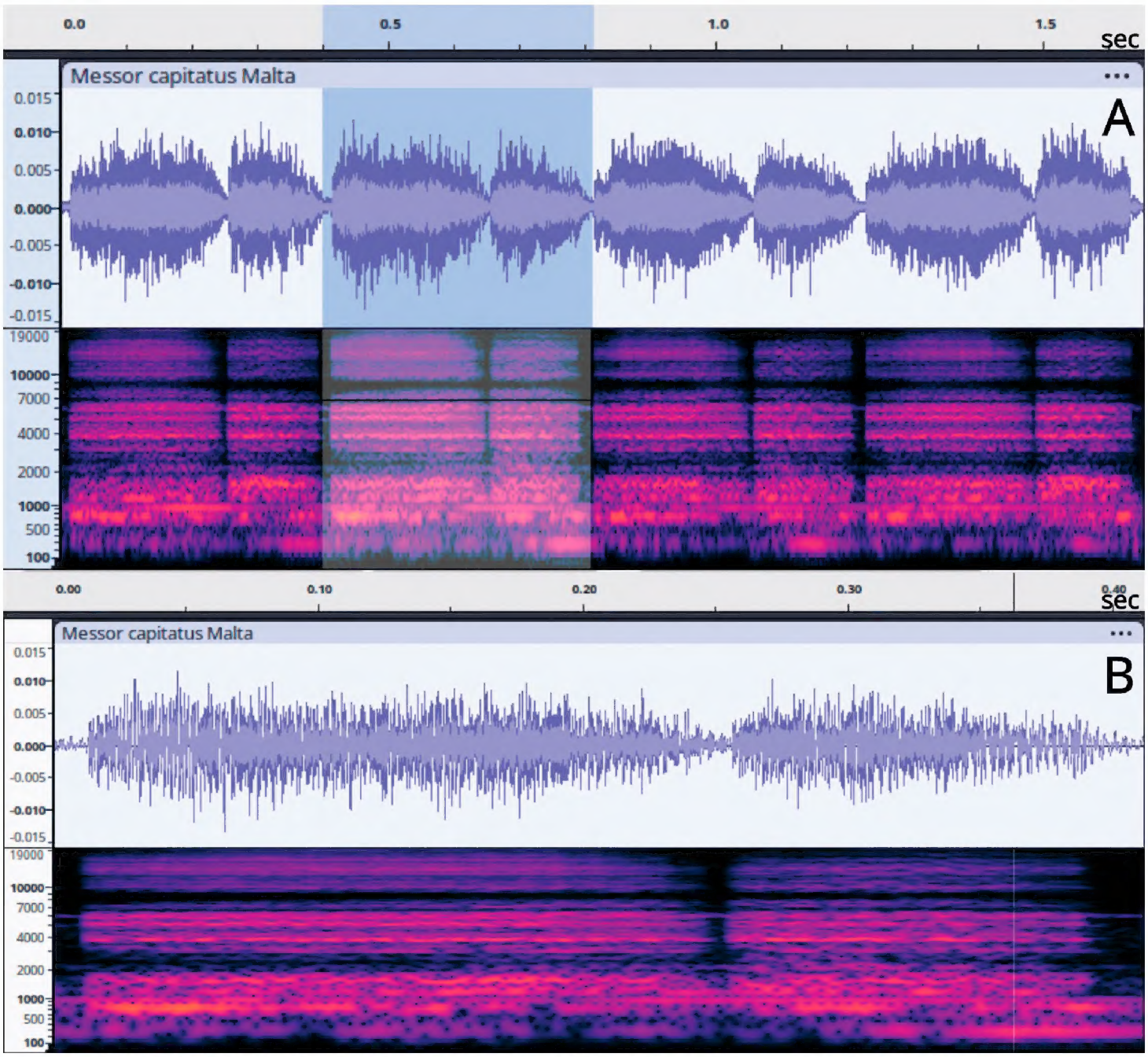

Figure 6. Oscillogram and sonogram of stridulation distress signals in *Messor capitatus* (worker) **A** train **B** chirp.

variation in acoustic parameters was observed in *M. bouvieri*, except for chirp rate, where *M. ibericus* showed the lowest variability. The greatest variation in the duration of both chirp parts occurred in *M. capitatus*, whereas *M. ibericus* displayed the highest variation for all other parameters (Table 1).

Acoustic characteristics of the studied *Messor ibericus* populations

The same parameters listed in Table 1 were used to characterise the acoustic signals of *Messor ibericus* specimens (major workers and a queen) from geographically distant populations from Malta, Gozo, Sicily, Crete, and Bulgaria (Table 2).

Acoustic parameters of *M. ibericus* major workers varied significantly among geographically distant populations ($F = 60.76$, $p(MC) < 0.001$), with post-hoc tests confirming differences between all pairs except the Malta-Bulgaria pair (Suppl. material 6: tables 5, 6). SDA identified chirp rate, backward frequency peak,

Table 1. Descriptives of the studied acoustic parameters of the three Maltese *Messor* species.

Acoustic parameter	Species	Sample size (N)	Range	Mean	Std. Deviation	Std. Error	Median	Coeff. of variation %
Chirp rate (chirp/s)	*M. bouvieri*	3	[2.09,2.48]	2.31	0.2	0.11	2.36	8.66
	M. capitatus	3	[2.25,3.01]	2.73	0.42	0.24	2.93	15.38
	M. ibericus	6	[2.85,3.15]	2.98	0.12	0.03	2.93	3.90
Chirp duration (ms)	*M. bouvieri*	9	[373,488]	428.22	31.44	10.48	430	7.34
	M. capitatus	9	[328,398]	359.78	28.31	9.44	360	7.87
	M. ibericus	18	[253,333]	295	26.53	5.79	293	8.99
Forward chirp duration (ms)	*M. bouvieri*	9	[155,217]	180.56	18.04	6.01	179	9.99
	M. capitatus	9	[109,227]	150.56	50.05	16.68	130	33.24
	M. ibericus	18	[151,220]	180	22.08	4.82	179	12.28
Backward chirp duration (ms)	*M. bouvieri*	9	[204,258]	236.11	18.2	6.07	245	7.71
	M. capitatus	9	[101,253]	197.67	63.04	21.01	218	31.89
	M. ibericus	18	[62,126]	96	16.92	3.69	99	17.58
Forward chirp frequency peak (Hz)	*M. bouvieri*	9	[3174,3284]	3229	39.9	13.3	3246	1.24
	M. capitatus	9	[3727,3893]	3777.89	68.86	22.95	3732	1.82
	M. ibericus	18	[2904,4036]	3387	397.02	86.64	3283	11.72
Backward chirp frequency peak (Hz)	*M. bouvieri*	9	[3167,3249]	3223.22	24.35	8.12	3228	0.76
	M. capitatus	9	[3301,3863]	3723.44	168.83	56.28	3741	4.53
	M. ibericus	18	[2900,4039]	3432	414.87	90.53	3601	12.09
Forward chirp amplitude	*M. bouvieri*	9	[-49.6,-44.7]	-46.84	1.47	0.49	-47.1	3.13
	M. capitatus	9	[-59.9,-52.3]	-56.83	2.66	0.89	-57.6	4.68
	M. ibericus	18	[-72,-49.6]	-62.04	5.63	1.23	-62.9	14.83
Backward chirp amplitude	*M. bouvieri*	9	[-56.1,-52.9]	-54.68	0.94	0.31	-55	1.72
	M. capitatus	9	[-64.6,-53.9]	-59.42	3.96	1.32	-58.2	6.66
	M. ibericus	18	[-75.6,-58.9]	-65.45	5.44	1.19	-63.2	14.31
Amplitude forward / backward chirp ratio	*M. bouvieri*	9	[1.13,1.22]	1.17	0.03	0.01	1.17	2.33
	M. capitatus	9	[0.99,1.13]	1.05	0.05	0.02	1.03	4.48
	M. ibericus	18	[0.94,1.19]	1.06	0.07	0.01	1.05	6.62

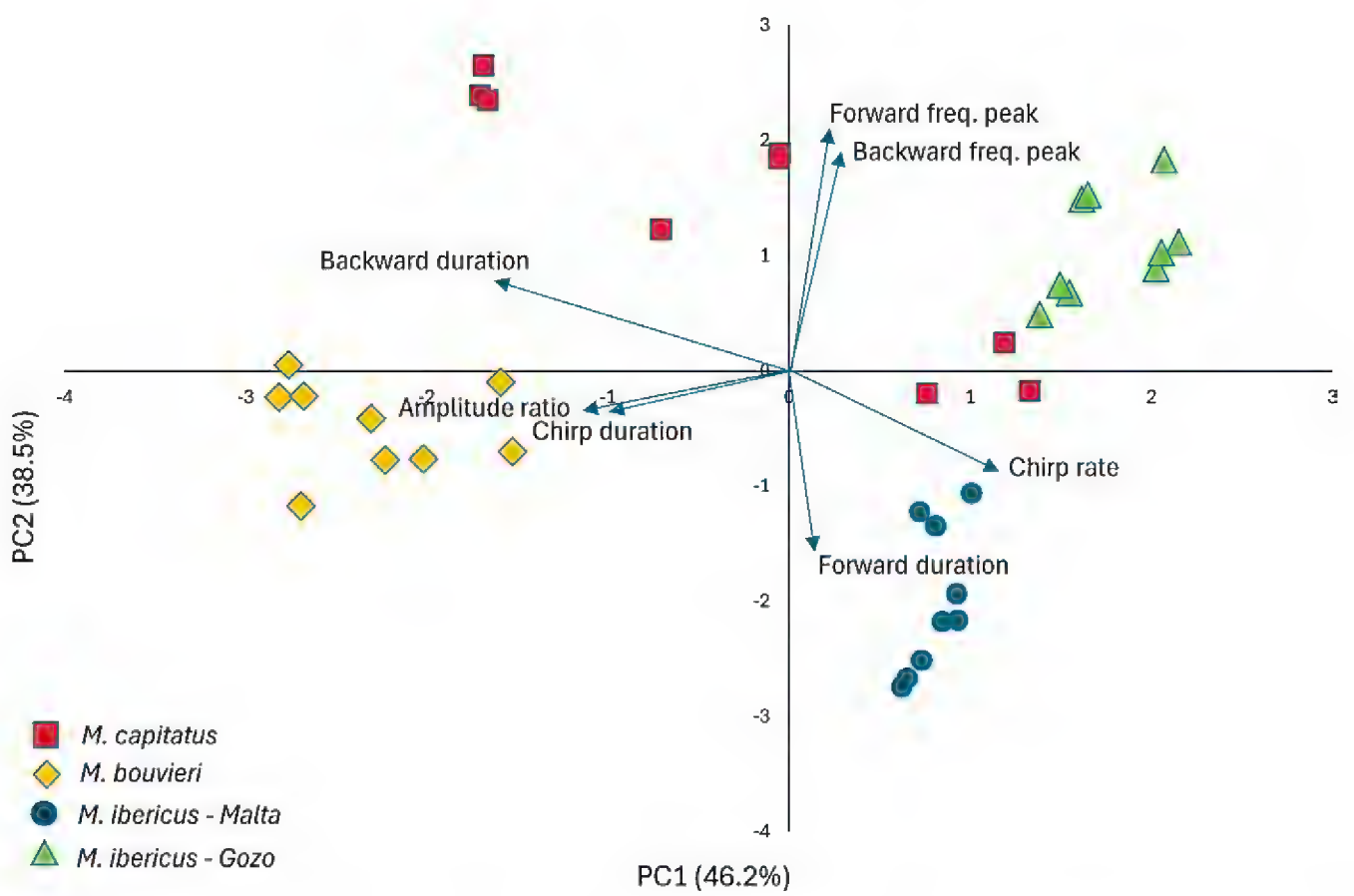

Figure 7. Principal Component Analysis (PCA) of the acoustic characteristics of *Messor* species from the Maltese Islands.

Table 2. Descriptives of the studied acoustic parameters of the *Messor ibericus* samples.

Acoustic parameter	Locality	Sample size (N)	Range	Mean	Std. Deviation	Std. Error	Median	Coeff. of Variation (%)
Chirp rate (chirp/s)	Malta	3	[3.06,3.15]	3.10	0.04	0.01	3.09	1.32
	Gozo	3	[2.85,2.93]	2.90	0.03	0.01	2.91	1.20
	Gozo queen	1	2.86	2.86	0	0	0	0
	Sicily	3	[5.74,6.88]	6.33	0.49	0.17	6.37	7.8
	Crete	3	[5.27,5.49]	5.41	0.09	0.03	5.45	1.73
	Bulgaria	3	[2.92,3.44]	3.19	0.22	0.07	3.22	7.02
Chirp duration (ms)	Malta	9	[286,333]	312.67	16.97	5.66	312	5.43
	Gozo	9	[253,314]	276.44	22.75	7.58	281	8.23
	Gozo queen	3	[277,331]	297.67	29.14	16.83	285	9.79
	Sicily	9	[153,171]	160.33	5.81	1.94	160	3.62
	Crete	9	[151,198]	169.89	15.93	5.31	169	9.37
	Bulgaria	9	[276,340]	302.78	21.48	7.16	301	7.09
Forward chirp duration (ms)	Malta	9	[161,215]	190.67	16.93	5.65	190	8.88
	Gozo	9	[151,220]	173.78	23.89	7.96	163	13.75
	Gozo queen	3	[151,190]	165.67	21.22	12.25	156	12.81
	Sicily	9	[76,86]	80.44	3.17	1.06	80	3.94
	Crete	9	[72,91]	81.78	7	2.33	82	8.55
	Bulgaria	9	[167,221]	191.33	19.97	6.66	195	10.44
Backward chirp duration (ms)	Malta	9	[95,126]	106.56	9.98	3.33	103	9.36
	Gozo	9	[62,111]	84.67	17.68	5.89	86	20.88
	Gozo queen	3	[90,111]	100	10.54	6.08	99	10.54
	Sicily	9	[67,91]	76.67	7.65	2.55	75	9.98
	Crete	9	[70,100]	78.56	10.88	3.63	73	13.84
	Bulgaria	9	[82,105]	96.11	7.04	2.35	97	7.33
Forward chirp frequency peak (Hz)	Malta	9	[2904,3307]	3027.78	180.17	60.06	2914	5.95
	Gozo	9	[3192,4036]	3646.22	328.07	109.36	3753	9.00
	Gozo queen	3	[3653,3709]	3683.67	28.38	16.38	3689	0.77
	Sicily	9	[3068,3288]	3165.44	74.17	24.72	3167	2.34
	Crete	9	[2984,3673]	3266.44	311.9	103.97	3057	9.55
	Bulgaria	9	[3074,3140]	3105.67	23.43	7.81	3101	0.75
Backward chirp frequency peak (Hz)	Malta	9	[2900,3296]	3019.89	173.40	57.80	2913	5.74
	Gozo	9	[3244,4039]	3779.33	241.91	80.64	3777	6.40
	Gozo queen	3	[3601,3678]	3628.33	43.09	24.88	3606	1.19
	Sicily	9	[2950,3227]	3123.11	90.74	30.25	3097	2.91
	Crete	9	[2966,3661]	3218.44	329.17	109.72	3015	10.23
	Bulgaria	9	[2753,3139]	3032.56	126.58	42.19	3092	4.17
Forward chirp amplitude	Malta	9	[-72,-49.6]	-59.90	7.87	2.62	-60.40	13.14
	Gozo	9	[-67.2,-61.1]	-64.19	2.35	0.78	-64.20	3.66
	Gozo queen	3	[-64.6,-59.6]	-62	2.51	1.45	-61.8	4.04
	Sicily	9	[-75,-57.8]	-67.39	7.01	2.34	-70.6	10.4
	Crete	9	[-55.5,-38.9]	-47.31	5.97	1.99	-44.7	12.63
	Bulgaria	9	[-63.7,-56.8]	-59.5	2.67	0.89	-57.9	4.49
Backward chirp amplitude	Malta	9	[-75.6,-59.00]	-65.74	6.56	2.19	-62.80	9.98
	Gozo	9	[-73.9,-61.3]	-66.96	4.13	1.38	-67.40	6.16
	Gozo queen	3	[-60.8,-58.9]	-60.07	1.02	0.59	-60.5	1.7
	Sicily	9	[-74.1,-60.6]	-69.09	4.54	1.51	-71.4	6.58
	Crete	9	[-66.2,-42.2]	-53.19	9.51	3.17	-49.4	17.88
	Bulgaria	9	[-65.1,-54.3]	-59.08	3.78	1.26	-58.1	6.4
Amplitude forward/backward chirp ratio	Malta	9	[1.02,1.19]	1.10	0.06	0.02	1.10	5.39
	Gozo	9	[0.96,1.11]	1.04	0.05	0.02	1.03	4.54
	Gozo queen	3	[0.94,0.96]	0.97	0.03	0.02	0.98	2.96
	Sicily	9	[0.95,1.16]	1.03	0.06	0.02	1.01	6.15
	Crete	9	[0.99,1.25]	1.12	0.07	0.02	1.11	6.68
	Bulgaria	9	[0.94,1.07]	0.99	0.04	0.01	1	4.04

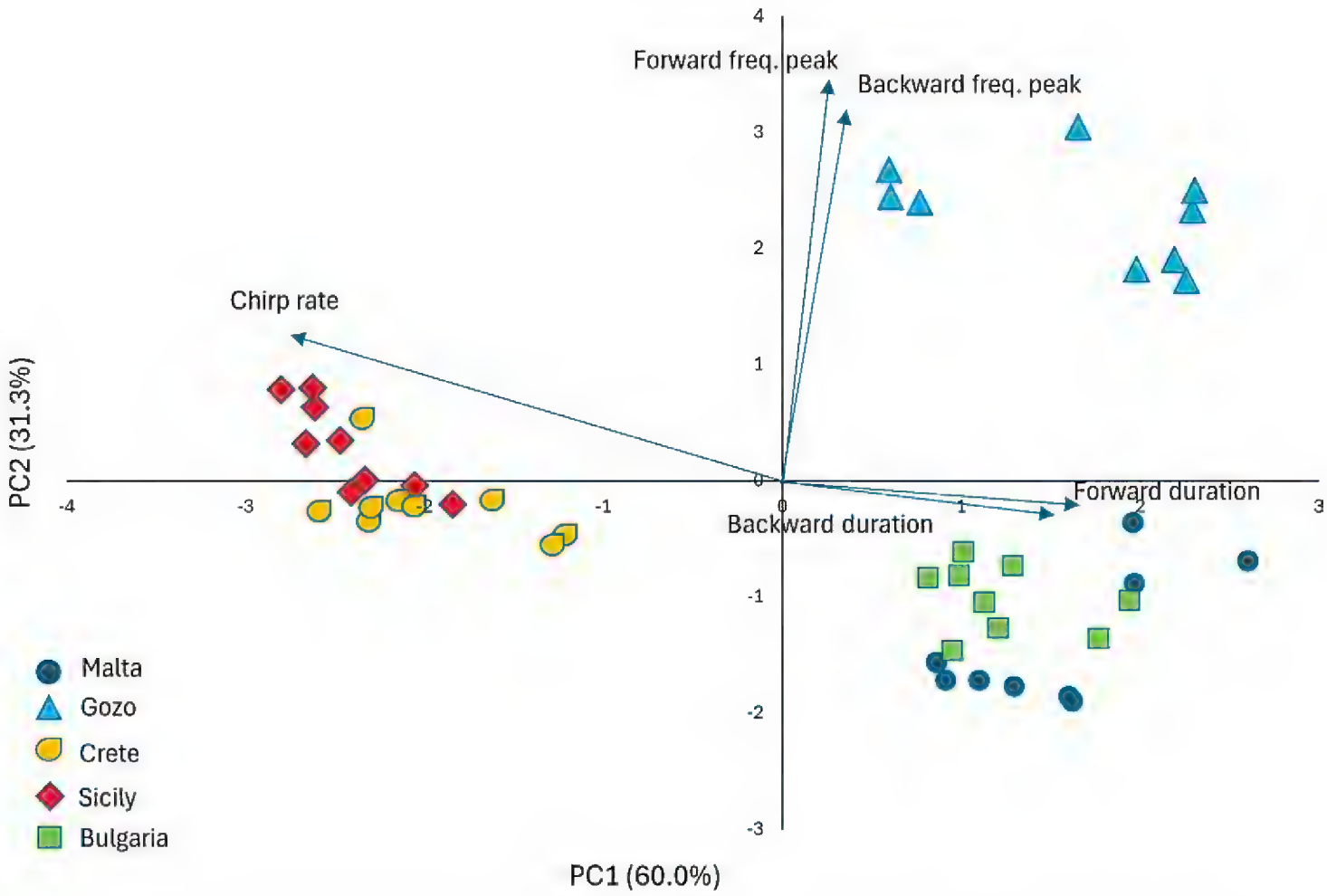

Figure 8. Principal Component Analysis (PCA) of the acoustic characteristics of *Messor ibericus* populations.

chirp duration, backward chirp duration, amplitude ratio between the forward and backward chirp, forward chirp duration, and forward frequency peak as discriminative variables (Suppl. material 6: table 7). PCA ordination (Fig. 8), consistent with the PERMANOVA results, placed the Bulgarian and Maltese populations in close proximity, reflecting their similarly long total, forward, and backward chirp durations—the longest among all populations (Table 2). However, the Maltese population showed markedly higher chirp frequencies, with signals from Gozo reaching the maximum values (3646.22 Hz in the first part and 3779.33 Hz in the second). In contrast, the Bulgarian population exhibited the lowest frequencies (3105.67 Hz and 3032.56 Hz, respectively). The Sicilian population displayed the highest chirp rate, followed by the Cretan population. The Cretan population also showed the highest ratio of forward- to backward-part amplitude (Table 2).

Discussion

This study constitutes the first investigation of vibroacoustic signals in ants from Malta and provides a comparative analysis of signals in workers of *Messor ibericus* across different geographical populations.

Workers of the three species possess a well-defined stridulatory apparatus, in line with previous reports on *Messor* ants (e.g., Grasso et al. 1998; Hernandez et al. 2002). Notably, only *M. ibericus* displays tegumentary foldings, the so-called "pillars", beneath the *pars stridens*, which are thought to play an amplifying role (Castro et al. 2015).

While several species of the genus *Messor* have been acoustically examined in previous research (Hahn and Maschwitz 1985; Baroni Urbani et al. 1988; Grasso et al. 1999, 2000), this study presents the first audio recordings of the three Maltese species—*M. capitatus*, *M. bouvieri*, and *M. ibericus*—together with oscillograms and analyses of their signal parameters. It is, however, highly probable that the stridulation data reported by Grasso et al. (2000) for *Messor structor* actually refer to *M. ibericus*, as their study predates the taxonomic revision of this species group (Steiner et al. 2018).

Although the sound recordings in Grasso et al. (2000) and those obtained in the present study both represent distress signals elicited by immobilization of *Messor* specimens with a thin pincer gripping the thorax, a direct comparison of the measured parameters is not feasible, as the studies employed different set and recording ranges—the ultrasonic spectrum in the former and the audible spectrum in the latter.

Our results demonstrate clear acoustic divergence among the three Maltese *Messor* species, though this should be interpreted cautiously given the small sample sizes. The data further enhances the nature of signal differences in studied species. *Messor bouvieri* consistently produced the longest chirps at the slowest rate, with the highest forward-to-backward amplitude ratio. Its signals were also the most stereotyped, showing the lowest variability across most parameters. In contrast, *M. capitatus* was distinguished by higher frequencies in both chirp parts and showed the greatest variability in chirp part durations, reflecting a more flexible or heterogeneous signaling system. *M. ibericus* exhibited the fastest chirps and shortest durations, with a longer forward part relative to backward one, which is the opposite to the pattern seen in *M. bouvieri* and *M. capitatus*. Notably, *M. ibericus* showed the lowest variability in chirp rate. These findings suggest that each species adopts a distinct signaling strategy. Such divergence may reduces acoustic overlap, especially in sympatric contexts where signal interference could hinder communication.

Further geographic comparisons reveal variation and significant differences within *M. ibericus*, with the exception of one pair—the Maltese and Bulgarian populations. Maltese populations exhibited chirp rates, similar to those of Bulgarian populations, but markedly lower than those of Sicilian and Cretan populations. Similarly, chirp durations were longer in the Maltese and Bulgarian ants than in the Sicilian and Cretan ants. Frequency peaks also varied geographically: Sicilian and Bulgarian ants produced lower values, whereas Maltese ants showed higher and more variable peaks, with the Gozo population presenting the highest frequency peak.

At the same time, parameters such as chirp rate and chirp duration may depend on various factors, including temperature and the individual's physiological state, as shown in crickets (e.g. Toms 1992; Ciceran 1994; Hartbauer et al. 2012).

Biogeographical and ecological notes on *Messor ibericus*

This study provides new insights into the presence of *Messor ibericus* in the Maltese Islands, specifically Malta and Gozo, where it was previously considered *M. structor*, and documents its habitat preferences and activity.

Prior to the taxonomic revision of the European *Messor structor* species group by Steiner et al. (2018), all records, including those from Malta, were attributed to a single

species, *M. structor*. Steiner et al. (2018) provided a new approach to species discrimination within the group, based not only on morphological traits, but also on geographical, genetical and ecological studies. To date, *M. ibericus* has been documented from Spain, France, Germany, Italy, Switzerland, Slovenia, Croatia, Romania, Bulgaria, and Greece, with its northernmost occurrence in Germany (50.05°N) and its southernmost presence in Crete (34.93°N) (Steiner et al. 2019; Salata et al. 2020). The new records from Malta represent some of the southernmost localities within the species' known distribution. According to Schifani et al. (2021), *M. ibericus* may be the sole representative of this group in Italy. While *M. structor* in southern Europe is the most cold-tolerant member of the group, occurring at elevations up to 1,800 m (Borowiec and Salata 2025), *M. ibericus* is more typically found at lower altitudes. Unlike the other two more common *Messor* species in Malta, *M. capitatus* and *M. bouvieri*, which were active during the day, the workers and queens of *M. ibericus* in Ta' Pinu (Gozo) were also active outside their nest at night.

New records of *Messor ibericus* corroborate the species' habitat preferences as it has been found in a dry valley in Malta (Wied Qirda), xerothermous grassland in Sicily, urban settlements in Plovdiv city centre in Bulgaria, garden in Crete and in crevices of sealed urban surfaces in Ta' Pinu in Gozo. Except for urban sites, all nests were situated beneath stones.

Conclusion

Although our sample size for stridulation recordings was limited, the observed patterns support the hypothesis that acoustic traits in *Messor* ants are subject to species-specific and population constraints. Future work with larger and more geographically diverse samples, along with experiments testing behavioral responses, will be essential for determining the function of these acoustic signals and clarifying whether population-level differences reflect variations in stridulatory organ morphology, stridulation behavior, or environmental influences on signal production.

Acknowledgements

We thank the Section Editor Francisco Hita Garcia and two anonymous reviewers for their valuable comments and suggestions, which helped to improve an earlier version of this manuscript.

This study was supported by the National Science Fund of the Republic of Bulgaria under grant No. KP-06-N-51/6, dated 11.11.2021.

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Supplementary material 1

Occurrence data on *Messor* ants from the study

Author: Albena Lapeva-Gjonova

Data type: xlsx

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Link: https://doi.org/10.3897/jhr.98.170415.suppl1

Supplementary material 2

Audio of stridulation performed by a worker of *Messor ibericus*

Authors: Albena Lapeva-Gjonova, Ilia Gjonov

Data type: wav

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Supplementary material 3

Audio of stridulation performed by a queen of *Messor ibericus*
Authors: Albena Lapeva-Gjonova, Ilia Gjonov
Data type: wav
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Supplementary material 4

Audio of stridulation performed by a worker of *Messor bouvieri*
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Data type: wav
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Supplementary material 5

Audio of stridulation performed by a worker of *Messor capitatus*
Authors: Albena Lapeva-Gjonova, Ilia Gjonov
Data type: wav
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Supplementary material 6

Results from statistical analysis

Author: Rumyana Kostova

Data type: pdf

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